Diapause in the Leaf Beetle *Diorhabda elongata* (Coleoptera: Chrysomelidae), a Biological Control Agent for Tamarisk (*Tamarix* spp.)

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ABSTRACT The tamarisk leaf beetle, *Diorhabda elongata* Brullé *deserticola* Chen, was collected in northwestern China and has been released in the western United States to control tamarisk (*Tamarix* spp.). Characteristics of diapause and reproductive development in *D. elongata* were examined to improve management as a biocontrol agent. Under long days, 16:8 (L:D) h, males began to emit aggregation pheromone within 2–3 d of adult emergence, mating occurred, and females oviposited within 7 d of adult emergence. Under short days, 12:12 (L:D) h, males did not emit pheromone, mating did not occur, and both males and females entered reproductive diapause marked by inconspicuous gonads and hypertrophied fat body. Ovaries of diapausing females lacked vitellogenic oocytes, and the ovarioles were clear and narrow, whereas reproductive females had enlarged ovaries with two to three yellow oocytes per ovariole. Diapausing males had thin, transparent accessory glands and ejaculatory ducts, whereas reproductive males had thick white accessory glands and white opaque ejaculatory ducts. Sensitivity to diapause-inducing photoperiods extended into the adult stage. Reproductive females ceased oviposition, resorbed oocytes, and entered diapause when switched from long to short days. Diapause-destined insects ceased feeding and entered the leaf litter 10–20 d after adult emergence, whereas reproductive insects remained on the plants and fed for at least 30 d. Reproductive insects exhibited dispersal behaviors, such as attempted flights, whereas diapause-destined insects did not show dispersal behaviors. Information gained from these studies will be used to better manage populations in the field and to improve rearing and storage in the laboratory.

KEY WORDS *Diorhabda elongata*, diapause physiology, dispersal behavior, tamarisk biocontrol, pheromone

Tamarisks (*Tamarix* spp.), also known as saltcedars, are comprised of several invasive weedy shrub species and their hybrids (Gaskin and Schaal 2002), which were introduced from Eurasia and Africa and have become a serious problem in the western United States (DeLoach et al. 2000). The invasion of *Tamarix* into riparian areas has caused substantial economic and environmental damage (Dudley et al. 2000, DeLoach et al. 2000, Zavaleta 2000, Shafroth et al. 2005) spurring development of a classical biocontrol program. Several promising control agents have been evaluated, but only the Eurasian leaf beetle, *Diorhabda elongata* Brullé *deserticola* Chen (Coleoptera: Chrysomelidae), has been released outside of quarantine (DeLoach et al. 2003, 2004).

During field trials, the leaf beetles have been shown to be effective agents in some locations where they have defoliated tamarisk plants, have overwintered, and have dispersed from the release site to distant stands of tamarisk (DeLoach et al. 2004). At other sites, however, the beetles have failed to overwinter either within field cages or in the open field and have had no impact on the target plant (Lewis et al. 2003, DeLoach et al. 2004). The success of the *Tamarix* biocontrol program will depend on achieving large and self-sustaining populations of *D. elongata* throughout the range of tamarisk in the western United States. To realize this goal, the basic biology and life history attributes of this insect should be used to understand population dynamics, developmental synchrony with the host plant, dispersal patterns, and ultimately to successfully manage populations of this species in the field (Chippendale 1982, Tauber et al. 1986).

Typically, insect phenology, behavior, and dispersal are closely tied to the nature and timing of diapause (Danks 1987), and this seems to be the case with *D.*

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elongata (Li et al. 2000, Lewis et al. 2003), making an understanding of diapause in this species critical to the goal of tamarisk biocontrol. Diapause is defined as a genetically determined state of suppressed development (Beck 1980), which also includes the expression of an array of behavioral and physiological adaptations, referred to as the diapause syndrome (deWilde 1962, Tauber et al. 1986). The specific nature of the diapause syndrome may be different from species to species, but it commonly includes delayed metamorphosis or delayed reproductive development, increased storage of metabolic reserves, decreased body water content, and diapause-specific behavioral patterns such as the cessation of feeding and the search for overwintering sites (Beck 1980, Tauber et al. 1986, Leather et al. 1993). The physiological changes associated with cold hardening may also be a part of the diapause syndrome (Denlinger 1991).

Members of the family Chrysomelidae commonly enter diapause as adults, although many species overwinter as eggs, or in some cases, they may overwinter in either life stage (Cox 1994). Initial observations indicate that *D. elongata* overwinter as adults in the leaf litter beneath the host plant (Lewis et al. 2003, Li et al. 2000). Females brought in from the field to controlled laboratory conditions, including long day photoperiods, oviposit for a longer time than females that remain in the field during late summer and early fall (Lewis et al. 2003). Other than these observations, diapause has not been described in this species.

At the onset of this study, a better understanding of diapause in *D. elongata* was needed for successful execution of the tamarisk biocontrol program. First, it was critical to have morphological markers of diapause so that the developmental status of field populations could be monitored throughout the season at different localities (Lewis et al. 2003, Spurgeon et al. 2003). Second, it was essential to know the life stage or stages during which diapause is determined. Such information, combined with temperature-dependent developmental rates (Herrera et al. 2005), is necessary for modeling population dynamics. Third, it was useful to know more about the behavior and pheromone biology of beetles in a reproductive, diapause-destined, or diapausing state to design effective monitoring, mass collecting, and release strategies. Recent identification of a male-produced aggregation pheromone in these beetles (Cossé et al. 2005) allowed this aspect of beetle biology to be included in this study. Finally, it was necessary to understand how to produce diapause insects in the laboratory for more efficient storage (Leopold 1998) and how to avoid diapause for maintenance of continuous cultures in the laboratory (Tauber et al. 1996).

The following report is a description of the diapause syndrome in *D. elongata*, concerned primarily with the morphological, behavioral, and pheromonal differences between diapause and nondiapause insects. This information is discussed in reference to managing *D. elongata* populations in the field and laboratory for more effective tamarisk biocontrol.

Materials and Methods

Insects. All *D. elongata* used in these studies were from a laboratory colony derived from beetles collected from three study sites in the western United States: Lovelock, NV (40°02′ N, 118°31′ W), Walker River, NV (38°53′ N, 118°45′ W), and Tinemaha Reservoir, CA (37°05′ N, 118°14′ W). All three sites have had beetles within cages since 1999, and at all three sites, beetles were released into the open field in 2001 (Lewis et al. 2003). Beetles at the three field sites were originally collected from an area in Xinjiang province near the town of Fukang (44°10′ N, 87°59′ E) in northwestern China (DeLoach et al. 2003, Lewis et al. 2003).

Laboratory Culturing of *D. elongata*. Larvae were reared in 473- or 947-ml polypropylene containers with ventilated lids. Reproductive adults were maintained in the 947-ml polypropylene containers. Pairs of adults were kept in 273-ml polypropylene containers. Later in the study, 2.8-liter plastic containers (Rubbermaid) with screen lids were used for mass rearing or storage of reproductive adults. These containers provide adequate ventilation and space for 50–75 developing larvae or 40–50 reproductive adults.

Larvae and adults were fed fresh cuttings of either *T. parviflora* de Candolle or *T. ramosissima* Ledebour, which were made into bouquets with cut ends in vials of water to preserve foliage quality. The insects feed and develop on either *T. parviflora* or *T. ramosissima*, which has been noted in host range studies (DeLoach et al. 2003, Lewis et al. 2003). When third instars ceased feeding and were ready to pupate, they were moved to containers with a 2- to 3-cm-deep layer of medium-grained sand into which they burrowed and pupated within loosely constructed sand cases. Emerging adults were immediately provided with foliage. Insects were cultured at 25°C under a photoperiod of 16:8 (L:D) h, which enabled continuous development or 12:12 (L:D) h, which induced diapause.

Defined temperature and photoperiod were maintained in growth chambers or incubators (Hotpack, Warminster, PA or model I30BLL; Percival, Perry, IA). Insects were weighed on a Mettler-Toledo AG104 (Columbus, OH) balance during the growth and feeding studies.

Photoperiod Switches. Insects were reared from eggs under conditions that promote reproductive development, 16:8 (L:D) h, 25°C, and groups were switched to diapause inducing conditions, 12:12 (L:D) h, 25°C, as second instars ($n = 14$), third instars ($n = 14$), prepupae ($n = 26$), pupae ($n = 16$), or newly emerged adults ($n = 24$). After adult emergence, the insects were paired, fed, and monitored daily for oviposition. Insects were considered to be in diapause if they never laid eggs. In another experiment, adults were paired and allowed to reach reproductive maturity under 16:8 (L:D), 25°C. They were divided into two groups, and one group was switched from 16:8 (L:D) h to 12:12 (L:D) h on the 10th day after adult emergence, when all of the pairs were laying eggs, whereas the control group was kept at 16:8 (L:D) h for

the duration of the experiment. All pairs were monitored daily for oviposition, and the switched beetles were monitored for an additional 10 d after cessation of oviposition in all of the pairs. Switched adults were dissected at the conclusion of the experiment to verify that they had entered diapause.

Pheromone Emission. Pheromone emission was measured from males destined for reproduction or diapause. For this study, larvae were reared under long-day 16:8 (L:D) h or short-day 12:12 (L:D) h conditions at the Albany, CA, laboratory, and after pupation were shipped by overnight express to the Peoria, IL, laboratory. On emergence there, adult males were placed into the pheromone collection apparatus with *Tamarix* foliage (Cossé et al. 2005), 10 males per collector tube. Of the insects reared under long-day conditions, 20 males were kept in the 16:8 (L:D) h photoperiod, and 20 males were transferred to the 12:12 (L:D) h photoperiod (in separate incubators, both at 27°C). The insects reared under short-day conditions were likewise divided between the two photoperiods (20 males per photoperiod treatment). Airflow through the collector tubes was 120 ml/min, and pheromone was captured from the air onto filters of Super-Q porous polymer. Collectors were tended (filters were extracted and foliage was renewed) daily for the entire study (>20 d). Beetles that died during the study were not replaced.

Pheromone analysis was performed by coupled gas chromatography/mass spectrometry. Quantitation of the extracts was done in selected-ion monitoring (SIM) mode, using 1-octanol as the internal standard (Cossé et al. 2005). Total pheromone [(2*E*,4*Z*)-2,4-heptadienal plus (2*E*,4*Z*)-2,4-heptadien-1-ol] emitted per male was calculated for each collection.

Observations of Behavior. Adult behavior was observed on live plants (*T. parviflora*) under conditions of controlled temperature and photoperiod in walk-in growth chambers (EGC, Chagrin Falls, OH). The plants were 40–55 cm tall, grown in 7-in plastic pots. Plants were covered with bridal veil mesh that was supported by a flexible frame of plastic-coated copper wire and formed a head space of 10–15 cm above the plant where beetles could freely move. The mesh covered the plants and came down over the base of the pots to allow beetles free access to a layer of dried tamarisk foliage, 2–3 cm deep on the soil surface. Experiments were initiated with the addition of five newly emerged and unfed adult pairs per plant. Treatments consisted of a 16:8 (L:D) h photoperiod or a 12:12 (L:D) h photoperiod; both were held at a constant 28°C. There were 5 replicate plants initiated simultaneously under the 16:8 (L:D) h photoperiod and 10 replicate plants under the 12:12 (L:D) h photoperiod, but these were initiated in two groups of 5 plants each on 2 separate d, designated 12L:12D (a) and (b). Observations were made three times per day, every other day, 3 d/wk, at 2–3 h after lights on (ALO), 6–7 h ALO, and 10–11 h ALO under both the 12:12 (L:D) h and the 16:8 (L:D) h photoperiods. Observations were made without disturbing the plants or beetles, and during each observation period, every beetle was located and categorized according to where it was found and what it was doing. The categories used to describe insect location included on the mesh in the upper 5 cm, on the mesh below 5 cm, on the stems, on the *Tamarix* trunk, on the leaves, or on the leaf litter. The categories used to describe behaviors included mating, feeding, sitting motionless, running, flying, and laying eggs. Plants were changed once during the course of the 12:12 (L:D) h treatments and twice during 16:8 (L:D) h treatment.

Categorical methods were used for the statistical analysis of dispersal behavior. The binary response variable for behavioral state was "dispersing," which comprised the behavioral categories of flight, attempted flight, and running on the mesh above the plant, versus "not dispersing," which comprised all other behavioral categories. Explanatory variables were time after lights on (three levels) and photoperiod (two levels). A logit model was used (Fienberg 1977): $\log(D_{ij}/N_{ij}) = c + t_i + p_j$, where D_{ij} is the number of beetles showing dispersal behavior at time i after lights on and in photoperiod j, and N_{ij} is the corresponding number of beetles not showing dispersal behavior; t_i is a parameter for the effect of time after lights on (i = 1, 2, 3, corresponding to times after lights on of 2–3, 6–7, and 10–11 h, respectively), p_j is a parameter for the effect of photoperiod [j = 1, 2, corresponding to photoperiods of 12:12 (L:D) h and 16:8 (L:D) h, respectively], and c is a constant. The customary constraints apply: $\Sigma t_i = \Sigma p_j = 0$. The model states that the ratio of dispersing to nondispersing beetles depends on the time after lights on and on the photoperiods but that the effects of time and photoperiod are independent. The likelihood ratio statistic for the overall fit of the model was $G^2 = 8.69$ (2 df, $P = 0.013$). The statistic indicates some evidence of lack of fit, caused by the interaction between time of lights on and photoperiod, but this interaction was very minor compared with the magnitudes of the main effects by themselves. The model fits well enough so that conditional tests (Fienberg 1977) for the main effects are considered to be reasonable. Further details are given with results. Calculations were done with Statistix software (Analytical Software 2003).

Dissection and Photography of Internal Morphology. Beetles were dissected in 200 mM NaCl in petri dishes with a clear silicone plastic pinning surface (BioQuip, Rancho Dominguez, CA). Most dissections were performed under an Olympus SZX12 dissecting scope and a fiber optic light source, and photos were taken through the scope with an Olympus DP11 digital camera. Measurements, including photo scale references, were made with a miniscale ruler (BioQuip; 5 mm divided to 0.1 mm).

The female reproductive system was described according to Büning (1994), although we designate the termini of the ovarioles as the germaria instead of tropharia. The male reproductive structures are described according to the terminology of Suzuki (1988).

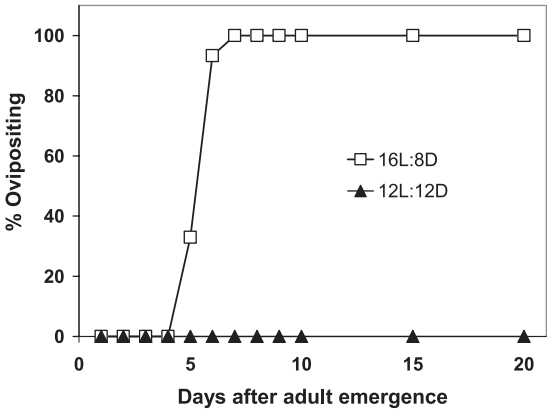

Fig. 1. Oviposition in *D. elongata* reared under long day (16 L:8 D h) or short day (12 L:12 D h) photoperiods at 25°C. Oviposition began 4–7 d after adult emergence under long days (n = 30 pairs) but did not occur under short days (n = 25 pairs).

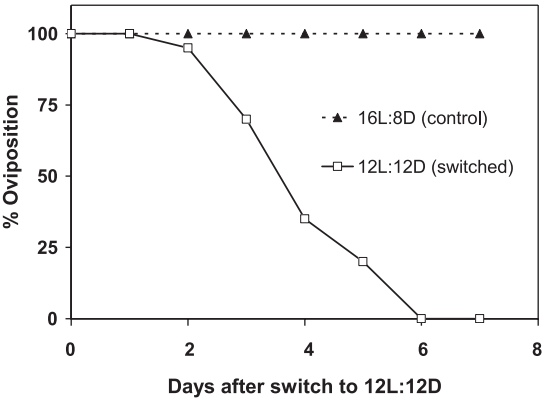

Fig. 2. Reproductive females cease oviposition within 6 d after switch from long to short days at 25°C. Pairs of adults (n = 20) that had been raised under 16:8 (L:D) h and had already laid eggs were switched to 12:12 (L:D) h on the 10th day after adult emergence. Pairs were monitored for 15 d after the switch. The control group (n = 9) consisted of paired adults that remained in 16:8 (L:D) h.

Results and Discussion

Induction of Reproductive Diapause. Pairs of beetles reared from egg to adult under short days 12:12 (L:D) h at 25°C failed to oviposit, whereas those reared under long days 16:8 (L:D) h at 25°C oviposited within 7 d of adult emergence (Fig. 1). In subsequent experiments at 25°C, we never observed diapause in insects reared under long days, and we never observed oviposition in insects reared under short days, although we observed oviposition at a very low level (<1% of females) under 12:12 (L:D) h, 28°C. Other life stages were not affected by photoperiod, and insects developed to adulthood at equivalent rates under both long and short days. These results are consistent with most reports that indicate that *D. elongata* overwinter as adults in reproductive diapause (Li et al. 2000, Lewis et al. 2003).

Continuous culturing techniques are valuable in the maintenance and amplification of populations of beneficial insects for biocontrol implementation (Tauber et al. 1996), whereas storage in diapause is efficient, convenient, and cost effective (Leopold 1998). In the case of *D. elongata*, a simple alteration of photoperiod determines whether the beetles will be in diapause or will be reproductive. This has allowed the production of diapausing beetles for long-term storage or the continuous culturing of reproductive insects.

Sensitive Stage for Diapause Induction. Beetles switched from 16:8 (L:D) h to 12:12 (L:D) h as second or third instars, prepupae, pupae, or newly emerged adults failed to oviposit. Subsequent dissection showed that both males and females had entered diapause without reproducing. From these data, it was clear that if insects had not reached reproductive maturity, they could be rapidly shifted to a diapause developmental program, entirely avoiding reproduction. In the case of insects that had already reached reproductive maturity and had begun oviposition, a switch to diapause inducing conditions put an abrupt halt to oviposition and rapidly reversed reproductive development (Fig. 2). Oosorption was noted in the ovaries of switched females, and hypertrophy of the fat body was noted in both males and females 20 d after the switch from 16:8 (L:D) h to 12:12 (L:D) h.

The sensitive period for reproductive diapause induction in *D. elongata* is indeterminate because it extends into the reproductive adult stage, as is the case with a number of insect species from at least five orders (Taylor and Spaulding 1988). Although there are numerous examples of insect species with indeterminate sensitive periods, most insect species have a determinant sensitive period in which information received earlier in life determines diapause later in life, and that decision cannot be reversed after a specific developmental point (Beck 1980). An indeterminate sensitive period gives *D. elongata* flexibility in responding to changing environmental (photoperiod) cues. Additional flexibility is gained through the speed of the response to photoperiod. After being switched from long days, only 2–5 short days are needed for previously reproductive females to cease oviposition and begin the process of oosorption. This remarkably fast physiological response may indicate rapid communication between the photoperiodic clock neurons and the endocrine system. Close association between clock neurons and the endocrine system has been shown in other insect species (Sauman and Reppert 1996, Siegmund and Korge 2001) and would be expected in *D. elongata* as well.

Internal Structure of Diapause and Reproductive Insects. There were no clear, distinguishing external features to mark diapause, so internal features were compared. Reproductive systems from both males and females were undeveloped in insects reared under short days, whereas fully developed reproductive systems were found in insects reared under long days (Fig. 3). The male reproductive system is typical of

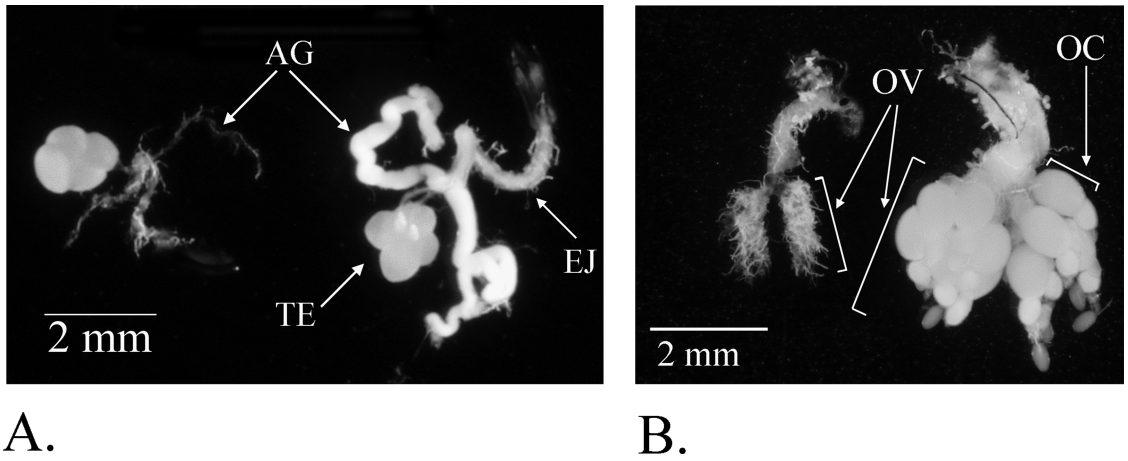

Fig. 3. Comparison of diapause and nondiapause reproductive systems. (A) Male reproductive systems showing diapause (left) and nondiapause (right) reproductive tracts. AG, accessory glands; EJ, ejaculatory ducts; TE, fused testes. (B) Female reproductive systems from diapause (left) and nondiapause (right) insects. OV, ovaries; OC, oocytes.

members of the subfamily Galerucinae (Suzuki 1988). Bright yellow to orange testes are fused into a single structure lying in the right side of the abdomen. The paired vas deferens connects the testes (TE) with the ejaculatory duct (EJ), and at the juncture of these structures is a single pair of elongate accessory glands (AG). In diapausing males, the AGs are transparent, thin, and inconspicuous, whereas in reproductive males, the AGs are conspicuous, thick, and filled with white fluid. The length of the AGs increases in reproductive males, but the most consistent changes are the increased thickness and opaque white appearance of the AGs in reproductive males (Fig. 3A). The EJ also increases in thickness and becomes opaque white in reproductive males.

The ovaries are paired structures consisting of 10 ovarioles per ovary, although as few as 8 ovarioles per ovary were occasionally found, and in one case, 12 ovarioles were found in an ovary. The ovaries are telotrophic, and individual ovarioles (OV) can be separated into the distal germarium containing the trophocytes and the proximal vitellarium containing the developing oocytes. The ovarioles terminate at the lateral oviducts, which merge into a common oviduct. The ovaries of reproductive females occupy a large proportion of the volume of the abdomen and contain bright yellow oocytes (Fig. 4). In diapause females, the ovaries are inconspicuous and enmeshed in tracheae (Fig. 3B). In both diapausing and reproductive females, the position of the ovaries within the abdomen is similar to that observed in the cereal leaf beetle (Wellso 1972), in which the terminal filaments are attached to the second thoracic phragma and the ovarioles attach to the lateral oviducts in the posterior half of the abdomen.

The ovaries begin as inconspicuous structures in newly emerged females, but after 5 d, they become the predominant abdominal feature in reproductive females (Fig. 3B). Growth and development of the female reproductive system in *D. elongata* was typical of the family Chrysomelidae (Büning 1994) and was divided into stages for ease of description. Stage 1

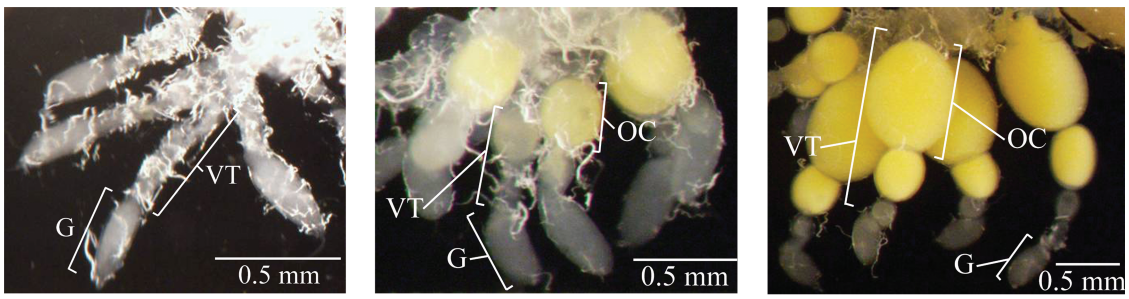

Fig. 4. Progression of ovarian development in *D. elongata*. Stage 1 ovaries are characterized by narrow, undeveloped germaria (G), <0.15 mm wide, and no yellow oocytes (OC) in the vitelleria (VT). Stage 3 ovaries are characterized by well-developed germaria, >0.15 mm wide, and vitellogenic (yellow) oocytes, 0.35 mm wide, in the vitelleria. Stage 5 ovaries are distinguished by having well-developed (0.7 mm wide) yellow oocytes with the proximal oocytes having chorion.

ovaries contain ovarioles, with the germarium approximately as thick as the vitellarium and lacking vitellogenic oocytes. This stage is typical of diapause females and newly emerged adults in early reproductive development. In stage 2 ovaries, the germaria are enlarged (>0.15 mm wide) and thicker than the vitellarium, which contains transparent previtellogenic oocytes. Stage 3 ovaries contain oocytes in the beginning stages of yolk deposition (<0.7 mm wide) and have well-developed germaria (>0.15 mm wide). Stage 4 ovaries contain oocytes greater than one half the width of mature oocytes (>0.7 mm), with large yolk deposits but no chorion deposition. Stage 5 ovaries contain full-sized chorionated oocytes and usually two additional developing (yellow) vitellogenic oocytes per ovariole. Reproductive females cycle between stages 4 and 5; stage 5 ovaries are found just before oviposition, and stage 4 ovaries are found after oviposition, before the next round of chorionogenesis.

In newly diapausing insects of both sexes, the fat body fills the abdominal cavity, whereas the fat body is often difficult to find in the abdomens of reproductive adults. The fat body of diapausing adults is made up of a mass of small, interconnected stringy lobes, whereas reproductive adults have small dispersed lobes of fat body. Diapause insects that have overwintered and have not recently fed may have depleted fat body stores along with undeveloped reproductive systems.

Reproductive insects switched to short days show reversal of development marked by atrophy and resorption of reproductive structures. In switched males, the accessory glands develop a roughened appearance and eventually shrink to become nearly the same in size and appearance as those of diapausing males that have never been reproductive (Fig. 3). Switched females retain oocytes of all stages, which are degraded and resorbed over a period of 20–30 d. Diapausing insects with well-developed fat body sometimes have the remnants of yellow oocytes retained in the ovarioles (Fig. 5). The appearance of the ovarioles and the degraded oocytes varies depending on the state of the ovaries at the time of switch, the stage of the oocytes being degraded, and the time elapsed since the switch. In switched insects, often the most proximal (most mature) oocytes are smaller than less mature oocytes (Fig. 5), which is different from the normal progression of oocyte maturation (Fig. 4). In other cases, the most mature oocytes appear to have chorion, but they are darker, ranging from dark yellow to orange-brown, and are misshapen with a collapsed appearance. The most distal oocytes have an irregular shape, differing from the round to ovoid shape of a healthy oocyte that has started to deposit yolk (Fig. 4). High variability makes ovarioles and oocytes in switched insects difficult to categorize, so all ovaries containing oocytes in the process of oosorption are referred to as regressing. Regressing ovaries have one or more of the following characteristics: (1) mature, proximal oocytes are smaller than, darker than, or more irregularly shaped than less mature oocytes; (2) terminal oocytes appear irregularly shaped with uneven yolk depositions; (3) oocytes have a dark yellow to orange-brown color and are irregularly shaped; (4) mature, proximal oocytes appear flattened or dented and range from dark yellow to reddish-brown, possibly with chorion deposition.

Fig. 5. Ovaries dissected from a previously reproductive female that had been switched from long day conditions to short day conditions. The ovaries show oocytes (OCs) that contain small amounts of yellow yolk but that are being absorbed in preparation for diapause.

Determination of Developmental Status Using Internal Morphology. The diapause status of *D. elongata* can be inferred by observing the condition of the reproductive system, combined with the level of fat body development. Suppressed reproductive development and hypertrophied fat body are typical features of diapause (Beck 1980, Tauber et al. 1986), but these features are sometimes subtle (Velarde et al. 2002). Fortunately, the contrast between diapausing and reproductive *D. elongata* is relatively high, presenting a good set of markers for determination of developmental condition.

Diapause females have stage 1, stage 2, or regressing ovaries, and they have a well-developed fat body. Diapause males have thin transparent accessory glands and a well-developed fat body. Males may have regressing accessory glands that are sometimes difficult to distinguish from fully developed ones. In such cases, the presence of a well-developed fat body indicates diapause. Reproductive females have stage 3, 4, or 5 (Fig. 4) ovaries and little fat body development. Reproductive males have thick, white accessory glands and little fat body development. Rarely, a small amount of fat body is found in individuals with fully developed reproductive systems, and in these cases, the insects are scored as reproductive. These criteria are similar to those used to distinguish diapause in the boll weevil (Spurgeon et al. 2003), although we made no distinction between different fat body types, and oosorption seems to be a clear indicator of diapause in *D. elongata*, in contrast to the boll weevil.

Some adults do not fall into either the reproductive or diapause category. They have no conspicuous fat body yet have undeveloped reproductive systems.

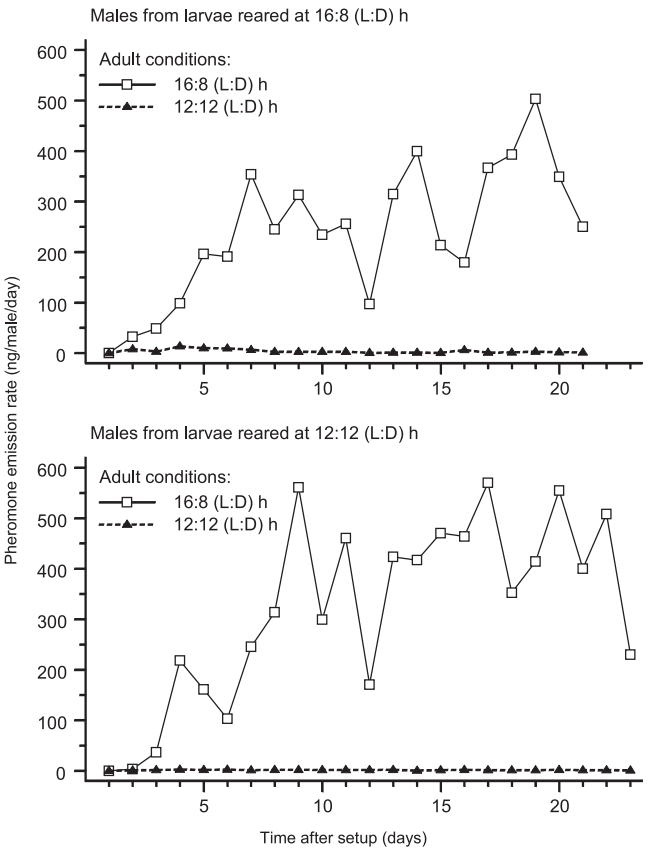

Fig. 6. Daily pheromone emission rates for adult males held under long-day (16 L:8 D h) conditions (solid lines) or short-day (12 L:12 D h) conditions (dashed lines). As larvae, these beetles experienced either long day photoperiods (top) or short day photoperiods (bottom). Each point is a mean for two collectors.

This condition is found in newly emerged adults and in adults that have overwintered and used most fat body reserves. The developmental fate of such individuals is unknown, so they are not counted in either category. Some field collections contain a high proportion of individuals that cannot be categorized, especially if the collection is made in an area with newly emerged adults (unpublished data). Despite this, it is usually simple to assess the developmental status of field populations because the morphological distinctions are usually clear. Using these criteria, we monitored the developmental status of field-collected and laboratory-reared populations.

The timing of diapause induction in the field is critical to the synchrony of insect life cycles with host plant availability (Chippendale 1982, Tauber et al. 1986), and the disruption of synchrony between diapause timing and crop plant availability has long been a part of pest management strategies (Chippendale 1982). Management of a beneficial species such as *D. elongata* presents the opposite problem, which is management for synchrony of diapause with host plant availability across a wide geographic area. To that end, the morphological characteristics presented here should be helpful in monitoring the developmental status of populations of *D. elongata* in the field.

Pheromone Emission. Males held under the long-day conditions began to emit detectable amounts of pheromone within 2–3 d of emergence, regardless of whether they experienced 12:12 (L:D) h or 16:8 (L:D) h photoperiods as larvae (Fig. 6). Pheromone emission continued until the study was terminated at 21 or 23 d after setup. Ratios of pheromone components were similar from day to day and between groups and were consistent with Cossé et al. (2005). However, pheromone emission from males kept under short-day conditions was negligible. Trace amounts of the aldehyde component were detected, but the levels were consistent with foliage damaged by feeding (Cossé et al. 2005).

Pheromone emission is considered part of the reproductive biology in these beetles, and the pattern of pheromone emission with respect to daylength corresponded closely to the patterns observed for reproductive activity and reproductive system morphology.

Reproductive and Diapause-Associated Behaviors. When newly emerged adults were allowed to feed and move freely on *T. parviflora* plants, their behavior patterns were photoperiod dependent. Under diapause-inducing conditions, 12:12 (L:D) h, 28°C, beetles fed heavily for the first 5 d of adult life, followed by a period of inactivity in which they remained on the

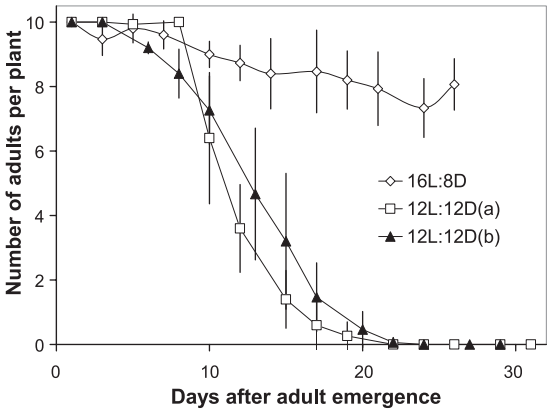

Fig. 7. Presence of adults on live plants under a 12:12 (L:D) h and a 16:8 (L:D) h photoperiod. Open squares and closed triangles represent the average number of adults found per plant, ±SD, under 12:12 (L:D) h, from two replicate trials (a and b). Open diamonds represent the average number of adults per plant, ±SD, under 16:8 (L:D) h.

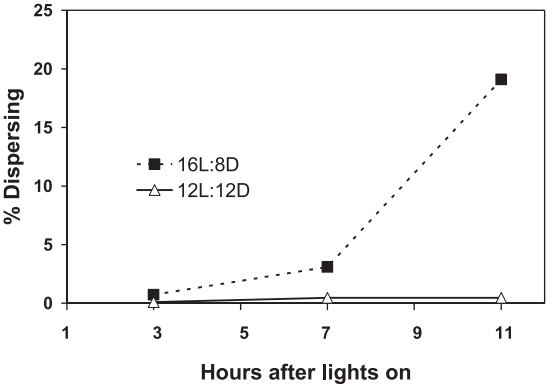

Fig. 8. Dispersal behavior versus time of day after lights on under long versus short days. Percentage of adults displaying dispersal behavior is shown for three times during the day and is a combined total observed during 11 d of the experiment. The 12:12 (L:D) h group represents the combined data from 10 plants, with 10 insects per plant, whereas the 16:8 (L:D) h group had 5 plants with 10 insects per plant.

foliage. By day 20, all insects had left the foliage and had descended into the leaf litter for the duration of the experiments (Fig. 7). Under 16:8 (L:D) h, 28°C, adults fed and were active for the entire experiment. Four days after adult emergence, mating was frequently observed, and after that, oviposition was observed for the duration of the experiment. The decline in absolute number of insects found on the plants under long days reflected mortality and not migration to the leaf litter. At the conclusion of the long day experiment, 8 of 50 insects were found dead (16% mortality), whereas no mortality was observed in the diapause groups. At the conclusion of the short day experiments, 99 of 100 insects were found alive, buried to a depth of 1–2 cm in the leaf litter. Apparently senescence and aging are slowed during diapause (Tatar and Yin 2001).

Feeding was observed in diapause-destined and reproductive adults. On day 3, feeding was observed a total of 60 times in the 100 beetles held at 12:12 (L:D) h but only 4 times in the 50 insects held under 16:8 (L:D) h. After day 3, observed instances of feeding declined dramatically in insects held at 12:12 (L:D) h, and no feeding was observed after day 10, even though some insects remained on the plants until day 20 (Fig. 7). In contrast, with insects held at 16:8 (L:D) h, feeding was continuous for the duration of the experiment and observed an average of nine times per day. In a separate experiment, wet weight gain was measured in diapause-destined and reproductive insects, and wet weight nearly doubled during the first 7 d of adult life under either treatment (data not shown). After that, diapause-destined insects did not gain weight, whereas the weight of reproductive insects fluctuated through ovipositional cycles.

Mating was frequently observed in the 16:8 (L:D) h treatment but never observed in the 12:12 (L:D) h treatment. Under 16:8 (L:D) h, a total of 229 mating pairs were recorded during 33 observation periods. Because 50 insects (25 pairs) were being observed in this experiment, there was the potential to see a total of 825 mating pairs during the 33 observation periods if all insects had been alive and mating during all observation periods. Because a total of 229 mating pairs were observed, the overall frequency of mating was 28%, but frequencies varied during the experiment and between observation periods. No mating was observed on day 3, when the most reproductively advanced females had only reached stage 3 of ovarian development (data not shown). Considering observations from day 5 onward, the morning observation period (2–3 h ALO) had the highest mating frequency at 45.2%, dropping to 32.4% in the early afternoon (6–7 h ALO) and only 14% in the late afternoon (10–11 h ALO).

Dispersal behaviors were observed significantly more frequently under 16:8 (L:D) h than under 12:12 (L:D) h (Fig. 8); conditional G^2 for differences between photoperiods was 222 ($df = 1, P < 0.001$). These behaviors were also observed significantly more frequently in the late afternoon, 10–11 h ALO, than in the morning or at noon (Fig. 8); conditional G^2 for differences among times of day was 150 ($df = 2, P < 0.001$). This corroborates observations at two Nevada field sites where reproductive adults were seen flying in the mid- to late afternoon, 9–13 h after sunrise, and very little flight was observed in the mornings (unpublished data). Flight was rare in the late summer when diapause-destined insects mass on tamarisk plants and descend into the leaf litter (unpublished data).

The behavioral program for reproductive *D. elongata* favors mating in the morning and dispersal flights in the mid- to late afternoon. In diapause-destined beetles the program is altered to favor fewer flights and no mating. In the field, this translates to active and sometimes dramatic dispersal flights in reproductive adults and an almost complete lack of flight in insects

that are massing on tamarisk plants in preparation for overwintering (unpublished data). The search for overwintering sites is a typical diapause associated behavior in many species where dispersal flights may take adults to sites distant from the host plants (Tauber et al. 1986, Danks 1987). Short-range diapause-associated dispersal behavior has been noted in some species (Voss and Ferro 1990), whereas spectacular diapause-associated migrations have been noted in other species (Dingle 1978). Such dispersal flights have not been seen in diapause-destined *D. elongata*, which have been found to overwinter directly beneath or very near to the host plant (Lewis et al. 2003).

Reproductive state and emission of the male-produced aggregation pheromones are both closely regulated by photoperiod (Figs. 1, 2, and 6), indicating an important role for aggregation pheromones in the reproductive biology of *D. elongata.* Reproductive beetles disperse in the afternoon and mass in the tops of tamarisk plants in large collections of mixed-sex adults (unpublished data). These aggregations can be very dynamic, forming and disbanding rapidly during the afternoon hours, but becoming stable by dusk. In the morning, the aggregations are stable, and large numbers of mating pairs are present. This pattern ceases as insects enter diapause later in the season. It seems likely that the aggregation pheromones play a role in calling in a mixture of males and females to suitable plants where mating pairs can form. The sequence of behavioral events and the role of other attractants in formation of these mating aggregations need further study to better understand reproductive biology in this species.

Information on the behavior of *D. elongata* has been incorporated into recommendations on the collection and release of beetles in biocontrol implementation. Our recommendations are that reproductive adults be released in the morning or at sunset to avoid immediate dispersal that would likely occur in the afternoon. Field collections of diapause-destined adults are best made in the early fall when they are massing on tamarisk plants in a state of relative inactivity, before descent into the leaf litter. Plants with large numbers of inactive, diapause-destined insects can be identified and used as sites for collection or monitoring of overwintering populations because insects stay near to the plants on which they complete feeding.

Using Diapause Studies in the *Tamarix* Biocontrol Program. We have described diapause in greater detail to better understand and use *D. elongata* as a tamarisk control agent. Although diapause has been the subject of study in hundreds of insect species (Beck 1980, Tauber et al. 1986, Danks 1987, Leather et al. 1993), relatively few weed biological control agents have been studied in detail (Tauber et al. 1996, Velarde et al. 2002). Information presented in this paper combined with other studies of basic biology (Lewis et al. 2003), development (Herrera et al. 2005), ecological host range (Dudley and Kazmer 2005), and chemical ecology (Cossé et al. 2005) of *D. elongata* have proven beneficial to the tamarisk biological control project and will continue to provide a biological basis for tamarisk biocontrol strategies.

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